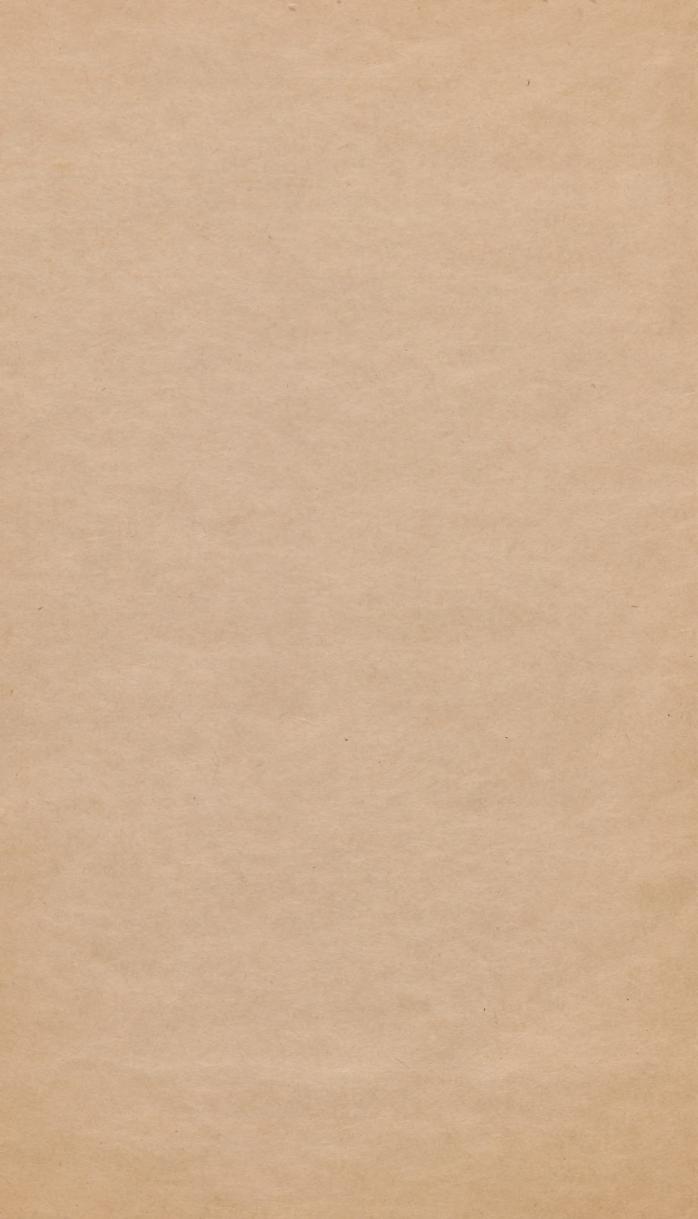
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"Experimentation with Typhoid Bacillus and Typhoid Vi Bacillus," by Epidemiology Laboratory of Army Medical College, 31 Mar 42





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Army Heilesl College Spidemiological Research Report Section 2 Number 327

Belationship of Supersonic Mave Frequency and Cellulisidal Action

Part 2. Experimentation with Typhoid Sacilius and Typhoid Vi Bocilius

Japan. Arm Medical College Epidemiology Laboratory (Maj. Gen. ISBII, Commanding) ENDO, Takechi Non-efficial staff

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- F. Observation of morphological changes.
- 9. Measurement of turbidity.

Chapter III. Results of experiment.

- A. Collulicidal time.
- B. Survival test.
- C. Observation of sorphological changes.
- D. Heasurement of turbidity.

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Bibliography

General

The numerous works of MATABABE¹, YES and SIE LIE², SAMADA³, CHARL⁴ and FUMATO⁵ contain accounts of the mechanical destruction of typhoid bacilli by means of a supersonic wave treatment and the resulting decrease in texins and increase in antigenic strength. However, the influence exerted on these bacterial cells by supersonic waves of varying frequencies but of the same energy level has not been reported.

My min was to further improve the quality of typhoid vaccines through a study of the destructive strength of supersonic waves of varying frequencies on the same antigen.

Chapter 1. Outline of experiment

Following the procedure in the cholera bacteria experiment in Fart 1 of this series, the survival, bacterial count, sorphological changes and turbidity of typhoid bacilli (Shadebata strain) and typhoid Vi bacilli (No. 27) were examined.

Chapter II. Experimental procedure

- A. Sactorial strain: The typhoid bacilli were obtained from the strain preserved by this laboratory; the Vi bacilli (So. 27) were contributed by Technician NOJIMA of the Infectious Diseases Research Institute. Both strains indicated uniform turbidity in bouillen; gas formation in lactose and dextrose vertical egar was negative; whey culture and milk congulation were negative; lead acetate agar culture was positive; indol reaction was negative; and the agglutination titer of the typhoid immune serum was positive at 6,400 times.
- B. Bacterial suspension: Numerous cultures of Vi bacilli were made since each 3 per cent agar culture (PH 7.4) produced only a small quantity of colonies at 37° G over a 20-hour period. After weighing, a 10 ag per oc solution was prepared with a physiological saline colution; this was diluted to 1.0 ag per oc and 0.1 ag per oc.

Each of the prepared bacterial solutions was cultured in bouillon and agar; tests for miscellaneous bacteria were conducted after preparing smear specimens. Bacterial counts were performed before each experiment since the same bacterial suspensions were not employed whomever the supersonic wave frequency was changed.

C. Supersonic wave treatment: As already related in Part 1 of this series, the plate current was regulated to produce a test tube temperature (internal) rise of 10° C per minute at each frequency in order to maintain a constant energy input into a test tube containing a specific volume of transfermer oil. The following currents were used to provide a uniform energy level:

	Plate current	Grid current	Plate voltage
1120 kg	460 ma	166 ma	3000 ₩
560 kg	550 ms.	130 ma	3000 ¥
280 kg	400 ma.	190 ma	3300 v

As in the preliminary tests on cellulicidal strength and as outlined in Part 1, the treatment time at 1130, 560 and 280 kc was 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 80, 60, 79, 90, 105 and 120 minutes.

- D. Qualitative tests on collulicidal strength: Two platinum loop portions each of the supersonic wave-treated bacterial suspensions were slant-cultured with agar and plate-cultured with bouillon and agar. After incubating at 37° G for 24 hours the results were evaluated as +++ , ++ , + and according to their growth.
- S. Bacterial count: The supersonic wave-treated bacterial colutions were diluted progressively ten times each with a physiological saline solution. One co of such was sixed well with agar (45° C) and allowed to solidify. Agar was added again in an assumt sufficient to cover the surface. Bacterial count was taken after incubating at 37° C for 24 hours.

Because the purpose of this test was to determine the number of destroyed bacteria, counts were taken on the central and also during the intermediate stage and the stage ismediately prior to destruction. The rate of time decrease compared to the bacterial count of the control was examined. In brief, the percentage of developed colonies per milligram in comparison to the central was determined.

- F. Observation of sorphological changes: Issediately following the supersonic wave treatment, a smear specimen was prepared with one platinum loop portion of the test solution. Staining with sethylene blue was accomplished when this was dried and fixed. Microscopic examinations were made in order to study the nature of the destroyed bacteria.
- G. Honsurement of turbidity: The bacterial suspensions were diluted according to the ratios shown below with a physiological saline solution and measured with a Pulfrichts photometer. The absolute turbidity was computed from the relative turbidity.

Bacterial solution concentration

Pilution

0.1 mg/1.0 ec

Stock solution

1.0 mg/1.0 cc

10 times

10.0 mg/1.0 cc

100 times

Chapter III. Results of experiment

The results are shown in Tables 1-6. The bacteria survival times are shown in Figures 1-6, the serphological changes in Tables 7 and 8 and turbidities in Tables 1-6.

Table 1. Test results on colimicade strength; encuival and turbidity of typhoid becilli troated with 1110 ke supersonic cover.

Flats weltage 3000 v; plate current 460 ma; grid current 165 ma. Date of experiment 15 spr 41. Room temperature 26° C. Claudy.

				l ag				Annual transport		1.	0 100	Construction and American American	and the second second				10,	.0 eve		
Time (min.)	Servival Emeteria	ek .	Archidity	Sectorial count - 0.1 ag (E)	compared to	Log 2	Time (sde.)	tervivel bacteri	-	Turbidity	Bacterial count -	Survival caspared to	Log 2	Time (Min.)	Survival		Surbidity			log 2
	Bouillon	Apar			control (%)	AND THE PROPERTY AND THE PARTY AND THE P	ent or to the Property land	[oull) on	Agar elset			60003			Bouillon	Agar		(2)	compared to control (%)	
	+++	111	0,0151	70.1 × 10 ⁶		7,6452	K	.+++	+++	0.141	701.2 × 10 ⁶		8.8452	K	+++	+++	1.092	7.012 × 10 ⁶		9.8452
1	- ++-	++	0.0161				1	+++	1+1	0.151				1	+++	+++	1.026			
2	*6	*	0.0177	/ ,			2	+++	+i+	0.360	The state of the s			2	+++	+++	0.803			
3	*	1/2	0.0195	0.62 x 10 ⁶	0.008	5.7924	3	+++	+++	0.166				3	+++	1+1	0.780			
4	1	*	0.0006				1.4	+++	+++	0.103	Le paración de la companya de la com			4	111	+++	0.747			
5	-	**	0.0218				5	+++	+++	0,195	Grand Control of the			5	+++	+++	0.685			
6	-	***	0.0252			-	6	+++	+++	0,306				6	+++	+++	0.658			17.4
7	*	46	0.01.7				7	++	++-	0.214				7	++-	++-	0.635			
8		4	0.0259				8	+	++	0.223				8	-11	++	0.613			
9	**	***	0.0265				9	*	*	0.227	9.75 × 10°	1.39	6.9890	9	++	++	0,602			1.
10	**	490	0.0268				10	*	10-	0,237	2.00 x 30°	0.200	6.3020	20			0.580			
							15			0.4127			The same of the sa	20		*	0.452	32.200 × 206	0.544	7.5912
		*					20		and the second	0.4015		edition		40	***		0.6320	0.9 x 10 ⁶	0.013	5.99.3
													The same of the sa	50	-	-	0.5465			1
						and the same of th								. 60	***	428	0.7857			
											The state of the s		and the same of th	75	-	-	0.3903			
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		The second secon			The second second				and appropriate to the second					and the same of th						
		ACCESS OF THE PARTY OF THE PART											State of Lines and							1
					1		1			1				The second second second second second			1		1	

Table 2. Test results on collulicidal strongth; survival and turbidity of typhoid becilli treated with 550 km supersonic saves.

Plate voltage 3000 v; plate current 550 ma; grid current 130 ma. Date of experience 4 May 41. Soom temperature 29° C.

	gracies y captures de la formación de la companya d	a to eviden across the accessorie to	gan dan ini layor da ganahara da baharara da isa	o.l mg	description of the second	e ya indeed gan laa su changu u cum na kitiin ni dhi kunay				1.0) mg						20.0) 100		
Ties (min.) Surviv		Surbidit	Gal mg	Survival	Log 2	Thus (min.) bactord	20 A	Turbidity	Dectorial count -	Survival concared	log i	Time (min.)	Survivel bectors		Purblidity	Bacterial count -	Survival compared	Log S
and the second s	Bouille	on Aga		(2)	control (%)		The second secon	Boullion	Agar slant			oontrol (S)		Version Annual Control of the Contro	Boulllan	agar alone			control (%)	Nues es
	-111	The second secon	0,01454	72.302 x 10 ⁶	7.8596	7.8596	Manufacture (1) and (1	+++	+++	0,10019	723.624 × 10 ⁶	no service exemples and	8.8596		+++	+++	1,054,05	7230±240 × 10 ⁶		9.6596
1		to de la constant de	0.01328		Description of the last of the	Name of the last o	The statement of the st	The state of the s	-	0.09905		Samueletanomenera	Special Control of Con	1	+++	+++	1.00366			
2	1	+	0.01215	on the state of th		The same of the sa	SAS.		and and a second	0.08867		American States and Americ		2	+++	+++	0.97040			
3		-	0,01199	restriction of the second of t	and the same of th	opin de la company de la compa	2		++	0.08421			- Anna -	3	+++	+++	0.97040			
4	1	-	0.01136				4		+++	0,08254	384,833 × 10°	46.774	8.5108	4	+++	: +++	0.86220	Philippinesses		
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6		-	200	A CONTRACTOR OF THE CONTRACTOR		Name of the last o	6	+++	1	0.07930		gar agailtíre na reason		6	+++	+++	0.70270			
7		- The state of the					-	+++	-	0.06915	and the same	adjunction of the second of th	and the same of th	7	+++	+++	0,65809	Transferance and the second se		
8		Consequences			7,0098	7,0098	- C		++	0.07529	204.349 = 200	Control of the Contro	8.2653	8	+	†††	0.60232			
9			and the second		0.100		9		-	0.06581	W. C.L. 2 200	10.312	7.8729	20	+++	H	0.68559	turn resident and the second s		
10			disease	0.296 × 206	0.409	5,4723	1.0		++	0,06358	Stranger or wes	404 \$ 2000	Carried Control	20	+++	+++	0.47405	3004840 × 20 ⁶	4.156	8.4773
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20					A Company of the Comp		1 30	1 +	-+-	0,08992			CONTRACTOR	40	+++	+++		41.620 × 10 ⁶	0.575	7.6193
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Table 3. Test results on cellulicial strongth; survival on turbility of aglade belili treated with 20 as now many year.

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	Couillon	eler Simis			castrol (- illion	i my Blant		1.07	emiral (A)			is and the	e sir		\$13	control (1)	
K	1-1-1	<u> </u>	0.01.17	71. FLM. N 10		702 1000	4	4-1-1	1-1-1	0.00015	$705 \cdot 3000 = 30^5$		6.0764	18	- frankroung	6-4-4-	0.9.57	HILLON M.		9.00
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	Souillon	alant		3-7	control				Translate.	01 ST		100	to control			C 41100	al nt		V=7	control ()	
Š	+++	+++	0.017	7.31 = 10		7.6637			+++	+++	0.153	23040 it 30°	*	F.0659	T.	1		1.134	7216. x 10 ⁰		9.0631
1	+++	+++	0.016				1		+++	+++	0.120				2.	4	1-1-1-	1.450			
2	++		C.GL.						++	++	0.135						+++	1.075			de de la constante de la const
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7	+	+	0.015				17		+	+	0.090				77	++	++	0.730			
	+	+	0.025	3.95 m 30 ⁵	204, 23	6.5266	0		+	+	0.00	Seld N.30"	1.007	6.0037	.0.	++	++	0.661			
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							And the control of th	many applications of the second					To Dispersion of the Park		20			0.717			
						Total Age	The state of the s			management minima (va			The state of the s		72	_	_	1.119			
								Annual (Control of Control of Con		Total Control					75		_	1.119			

Table 5. Test results on cellulicidal strength; survival and turbidity of trabel Vi L cilli trented with 560 ke supersonic waves.

Flate voltage 3000 v; plate current 550 ma; grid current 130 ms. Date of experient 2 - 5 Jun 41. Loom temperature 260 .

A Application to the second	- The second of the second		0.1	l ng						1.0) mg	,					10.	o ng		
Time (min.)	Surviva. bacter:		Turbidity	Sacterial count - C.1 mg	Survival compared	Log	ile (mlr.	bacter:	l of la	Turbidity	Encionial count -	compared	Log L	Nie (mln.)	arviva bacter	l of ia	furbidity	Dacterial count -	Survival compared	Log Z
	Bouillon	ver slant		(#)	to control			Bouillon	alent	per la constanta de la constan	(2)	to control			Douillon	/car		(=)	control	
K	-+++	++-	0.01174	72.447 × 10 ⁶		7.8599	5		+++	0.01929	735.457 x 10 ⁶		8.8599	2	+++	+++	0.83655	7244.79 × 10 ⁶		9.8599
1	+1+	-++-	0.01126				American description of the second se	+++	+++	0.02700				2	+++	+++	0.80909			
2	+++	+++	0.01.226			A photography (see page 1).	6 6	+++	+++	0.07919				2	+++	+++	0.73759			
3	++	+	0.01312			WEI THE PROPERTY OF THE PROPER	Y	+++	+++	0.07752				3	+++.	+++	0.59116			
A	++	++	0.01360			National Control of the Control of t	br	+++	+++	0.07585				h	+++	+++	0.63570			
5	++	++	0.01382	39.357 × 10 ⁶	54.326	7.5949	The second secon	+++	+++	0.07562	217.147 × 10 ⁶	29.973	8.3367	5	+++	+++	0.64(93			
6	-++	++	0.0130			ad it continues the	and the state of t	+++	+++	0.06748				6	+++	+++	0.68039			
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8	+	+	0.01583	6.683 x 30 ⁶	9.25	6.0050	A C	++	++	0.07473	117.56 x 10 ⁶	10.228	8.0701	8	+++	+++	0.60232			
9	+	+	0.01582				1	++	++	0.07429	A Commence of the Commence of			9	+++	+++	0.574.3			
1,0	+	+	0.01560	0.115 x 10 ⁶	0.160	5.0607	30	++	+	0.06525	49.175 a 20	6.004	7.4778	10	++ /	++	0.58001			
15	+	+	0.013/6	0.33 × 106	0.0.6	4.5105	15	++	+	0.06915	39.171 × 10 ⁶	5.448	7.59/3	20	++	++	0.47962	274.83 x 10 ⁶	3.793	8.4391
20	1-1	100000	0.012675			to the statement of the		++	+	0.00693	33.519 = 10 ⁶	4.63	7.5305	30	++ ;	++	0.41/116	15.00 × 10 ⁶	0.200	7.1761
						Andreas a property of the state	30	400.	-	0.07017	Total and a second a second and			40	++	++	0.44951	11.46 × 10 ⁶	0.162	7.0705
							40	_		0.061347				50	++	++	0.0831	6.03 x 10 ⁶	0.085	6.7784
									man in the second secon					60	++	++	G.33347	2.55 x 10 ⁶	0.035	6.4005
														75	+	+	0.33673	0.13 x 10 ⁶	0.002	5.1139
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Table 6. Test results on cellulicidal strength; curvival and turbicity of typhoid Vi Leill treated at 1 10 to experience area.

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1	-+++	+++	0.01487	65.935 × 10 ⁶	90.107	7.0291	2	+++	+++	0.00177	77.710 : 17	10.893	7.9016	1	+++	++-	0.8110			And a second sec
2	+++	+++	0.01253				. 2	+++	+++-	0.750				2	+++	+++	0.6469			
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A. Collulicidal time.

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The results show an acceleration in cellulaidal time (in the order of 360 hc and 1120 kc) with higher besterial solution dilutions at the same an erecals wave energy level. Destruction, herever, is reliable at 500 kc. The cellulaidal times for the typical bestill at 200 kc are six sinutes for G.I mg. 15 minutes for 1.0 mg and 40 minutes for 10.0 mg. At 1120 kc the time for 0.1 mg is four minutes or a secondar faster rate than that at 250 kc; the other times are alsower. The times for the Vi bestill at 250 kc are nine minutes for 0.1 mg. M minutes (15 minutes at 1120 kc) for 1.5 mg and 50 minutes for 10.0 mg. Consequently, the destructive strength at 250 kc is directly preparticulate to the frequency and inversely proportionate to the wavelength and is subject to some assumt of fluctuation.

a superiod test: (See Figures 1 to 6) The graphs show that a superiod news treatment produces a logarithmic decrease in bacterial counts in the graphs as logarithmic somics (vertical spee) and the treatment times as arithmetical scales (norisental axes), superximately straight lines are forced. A step-lies decrease is indicated by the 10.0-by concentration. By treating the bacterial count as y and the treatment time as a, the relationship

log 7 s or, or

FRE ex

(c . negative constant)

is palained.

in illustrated by the besterial counts for the 18.0 mg/l.0 co contentration in rigures 3 and 6, the decrease in live besteria count, at the mass energy level, becomes more pronounced as the frequency accreases or, in other words, as the wavelength increases.

The time required for the log of the live bacteria count of the 10.0 mg/1.0 or communicate to become helved is shown below.

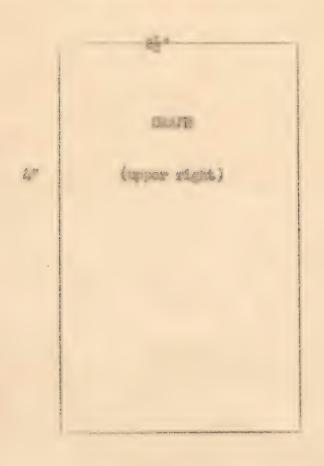
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Figure 1. Rectorial count variations of 6.1 mg/1.0 cc bacterial (typhoid bacilli) solution

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- 2 spok solution.
- 3 Time (minutes).

Figure 2. Bacterial count variations of 1.0 mg/1.0 cc bacterial (typhoid bacilli) solution



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- l factorial count.
- A stock solution.
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Figure 8. Pacterial count variations of 10.0 mg/1.0 cc bacterial (typhoid bacilli) solution

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- 1 Esciental count.
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Figure 4. Basterial sount variations of 0.1 ng/1.0 os testerial (typhoid Vi basilli) solution

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- 1 meterial count.
- 2 Stock colution.
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Figure 5. Easterial count variations of 1.0 mg/1.0 oc bacterial (typhoid %1 bacilli) solution

- l Bacterial count.
- I Stock solution.
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Figure 6. Eneterial count variations of 10.0 mg/1.0 co bacterial (typhoid Vi bacilli) solution

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- 1 Sectorial count.
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- 6. Observation of surphological changes:

Table 7. Dicroscorie descriptions of comphological changes of symbold bacilli at various and the first marrier

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Table 8. Microscopic observations of morphological change in typhoid Vi bacilli at various supersonic wave frequencies

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It was learned from the foregoing results that the optimum for callular destruction is at 280 kg for both types of becilli.

D. Heasurement of turbidity: As shown in Tables 1 to 6, transparency was not observed among suspensions of typhoid becilli within the superconic wave frequency range covered by this experiment. An extremely slight turbidity decrease was detected in the 10.0 mg Vibacilli but changes in the others seemed to be non-existent.

Chapter IV. Summary and conclusions.

The following conclusions were derived upon observing the cellulicidal time, besterial count and turbidity and upon making microscopic studies on cellular destruction of typhoid becilli and Vi becilli, the suspensions of which were treated on the same energy level with supersonic waves at frequencies of 1120, 560 and 260 kg.

- A. At the same supersonic wave energy level the most effective cellulicided time is indicated at 280 ke and 1120 ke, in that order. Cellulicide at 560 ke is slow. This action is directly proportionate to the frequency (inversely proportionate to the wave length) in concentrated bacterial solutions and appears to be subject to sens amount of fluctuation.
- D. A slightly greater amount of difficulty is encountered in destroying the Vi bacilli compound to the typhoid bacilli.
- C. The callulicidal time decreases as the bacterial concentra-
- D. The changes produced in live bacilli, when based on treatment time, can be expressed legarithmically and pessess roughly the following relationship:

log y = ex.

y a live becteria count

x = trantment time

c = negative constant

- E. A microscopic examination of the specimene reveals cellular destruction occurring at 260 kg, 1120 kg and 560 kg, in that order.
- F. Transparency does not results when physiological saline solution suspensions of typhoid basilli and Vi basilli (only a slight trace can be noticed in the case of 10.0 mg Vi basilli) are treated with supersonic waves. So noticeable changes in turbidity are produced.
- G. Cellular destruction cannot be gauged by changes in turbi-

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I, D. NO.__ WDGS - INTELLIGENCE REPORT SUBJECT: Experimentation with Typhoid Racillus and Typhoid Vi Bacillus FROM: P/Y G-2, GHO, FEC REFERENCES: DATE OF INFORMATION: 1942 Source Former Japanese Army INCL ONG PREPARED BY: ATIS, G-2, GBO, FEC. Forwarded herewith four (4) copies Allied Translator and Interpreter Section Document No. 55388B, "Experimentation with Typhoid Bacillus and Typhoid Vi Bacillus" by Epidemiology Laboratory of Army Medical College, 31 March 1942. This report concerns experimental tests, which were made in the laboratories of the former Japanese Army Medical College in 1942, to improve the quality of typhoid vaccines. The study outlines the nature of the experiments, discusses experimental procedures and results and gives a summary and conclusion. For the Assistant Chief of Staff, G-2: J. H. POLK Lt Col. GSC 1 Inclosure (4 cys) Executive as mentioned above NOTE: This document contains information affecting the national defense of the United States within the meaning of the tents in any manner to an unauthorized person is prohibited UNCLASSIFIED Espionage Act, 50 U. S. C.31 and 32, as amended, its trans-CCS FORM 17A mission or the revelation of its conla relation Cover letter received the Res & S